

SEED COAT ANATOMY AND TAXONOMY IN EUCALYPTUS. I.

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(Plate i; nineteen Text-figures.)

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Synopsis.

Anatomical and histochemical investigations of the seed coat of Blakely's two Renantherous Sections of *Eucalyptus* aimed at revising these groups show for the bulk of the species a remarkable consistency. The seed develops from an anatropous, bitegmic, crassi-nucellate ovule. The salient testa characters are: the outer integument is several layered, with its outer epidermis built up by sclereids, its inner epidermis crystal-bearing but without forming a typical "crystal epithelium"; the inner integument persistent, two-layered and suberized. An elaborately ramified vascular system extends from the basal hilum to the distal chalaza.

However, three of Blakely's series, Ochroxylon, Steatoxylon and Myrtiformes, deviate considerably from each of these testa features and vascularization pattern and probably are not correctly placed in the Renantherae.

INTRODUCTION.

Since Bentham's (1866) original treatment the taxonomy of *Eucalyptus* has been based on various characters of the anther, such as shape, mode of dehiscence, attachment to the filament and relative position of the gland. It has been obvious more recently that some of the groups formed on this basis are not homogeneous. It has now been found that the anatomy of the seed coat, together with some morphological ovule and seed characters, promises to give valuable data for a regrouping of some species.

The value of the study of seed coat anatomy is not confined to its application to taxonomy, since it is likely also that seed coat structure and seed behaviour are related.

Blakely's "Key to the Eucalypts" is the most recent classification of the genus, and undoubtedly the most satisfactory yet published. In such a large genus, however, it is to be expected that even in so excellent a work there will be anomalies in the arrangement of some of the species, and at different times some of these have been suggested by investigations carried out in other ways. For example, Blake (1953) has shown on general morphological grounds how the species belonging to the Clavigerae as understood by him form a natural group, but in Blakely's "Key" they are partly separated. It has also been suggested (on the grounds of cotyledon shape) that some species having bisected cotyledons and separated by Blakely in his "Key" would more appropriately be placed together in the same taxon (Pryor, 1956).

Amongst all of Blakely's groups, however, the combined group Renantherae and Renantherae-Normales, containing about 100 species, is one which possesses a high degree of homogeneity, and it seems clear that this group, largely as defined by Blakely, might well be constituted a subgenus.

It was already known at the time this investigation began that in morphological characters the seed of the great majority of species within this composite group had features in common but at the same time these were to be seen seldom in species placed by Blakely in other sections. It seemed likely, therefore, that this group would be essentially a natural unit. It followed, therefore, that if a similar uniformity were found in seed coat anatomy this would conform with this view. Further, if among the species thus placed together by Blakely occasional exceptions to the common pattern of seed coat structure were found, it might be deduced that these were incorrectly included in the group. At this point, therefore, it appeared that an examination of seed coat structure would be profitable and the study was commenced.

The seeds used in this investigation were mostly the result of our personal collection, but some which were either rare or difficult to collect were obtained from different State Forestry Departments, Botanic Gardens and Herbaria, to which we are indebted for generous assistance. In a few cases seed from commercial sources has been used.

The microscopic sections were made by hand without prior preparation, and the drawings with a camera lucida.

Blakely (1955) has been followed in nomenclature and systematic grouping.

The purely anatomical aspects of the investigation considerably extend our knowledge in this field of research on *Eucalyptus*. They have been linked with previous publications on the subject, not all of which have been fully correct. There has been no previous reference in the literature to the taxonomic implications of seed coat anatomy in *Eucalyptus*, and the conclusions in this regard are arrived at entirely from the present work.

In the following, "testa" is taken to mean the seed coat derived from both seed integuments. Published work on the anatomical structure of the *Eucalyptus* testa has up to the present been quite limited. In an extensive work, Petit (1908) investigated the structure of 14 genera of Myrtaceae, including 59 species in the genus *Eucalyptus*. However, he gave emphasis principally to the anatomical structure of the fruit wall, both torus and pericarp, rather than to the seed coat. Only in 10 species was the testa also examined, and of these four species alone were illustrated. One of these, *Eucalyptus macrocarpa* (Fig. 22, page 51) is erroneous, illustrating, most probably, the testa of one of the species of the Renantherae.¹ The remaining nine species all belong to the Section Macrantherae, so that Petit's work does not give a balanced presentation of the distinct diversity of testa structure in the genus *Eucalyptus*. Raphe, chalaza and the course and structure of the conducting tissue were not investigated. Nor were the wall substances microchemically tested, so that the very interesting presence of an inner cuticle and of suberized tissues in the seed coat (with their physiological implications) escaped him.

Netolitzky (1926) gives a detailed and penetrating summary of seed structure for any work dealing with seed anatomy. The author records all facts known at that time and adds the results of his own critical studies. In particular with regard to *Eucalyptus* he reports generally the results of Petit's work and reproduces also, amending the annotation, three of his figures, *E. calophylla*, *E. globulus* and *E. "macrocarpa"*. Harada (1956) has examined in no great detail ten *Eucalyptus* species, but inaccuracies of observation and conclusion impair the value of the publication.²

In the fundamental works of Ferdinand von Mueller (1879) and J. H. Maiden (1929), figures and descriptions of seed are presented purely from the morphological viewpoint. Maiden gives a classification of seed according to colour, size, surface structure, position of hilum and similar features. Some of these characters are better understood, as will be seen later, if interpreted anatomically.

In addition we shall refer to other specific relevant points in the literature as they arise.

Finally it may be said that, no matter how important the role of anatomy may be in providing clues for taxonomic relationships, we are conscious of the fact that anatomical features alone (and from one part of the plant only) cannot lead to final conclusions until they are supported by evidence, especially morphological, from other parts. However, characters like testa structure and the vascularization pattern of the seed are features fixed by deep-seated factors of inheritance and scarcely affected by environmental factors. Therefore they may provide valuable facts for the establishment of taxonomic affinities.

¹ This view results from the study of seed collected ourselves from *E. macrocarpa* growing naturally near Wagin, Western Australia.

² Harada appears to be unaware of the precise delimitation of the two integuments. Several times he presents only the outer epidermis as the outer integument, and designates in text and figures the subepidermal parenchyma of the outer integument as the inner integument. In surface sections he speaks of a "network of strips" without realizing that these are cross-sections of anticlinal epidermal cell walls.

SECTION RENANTHERAE-NORMALES.

*The Outer Integument of the Ripe Seed.*³—This is composed of several layers. The outer epidermis is made up of a solid layer of sclereids with secondary, lamellated thickenings on the outer and radial walls, the latter traversed by corresponding simple or ramiform pit canals. The excessively centripetally thickened walls leave a very small lumen. They are mostly lignified, and this very strongly so in *E. triantha*, *E. laevopinea*, *E. macrorrhyncha*, *E. alpina* and others, but no phloroglucin reaction was obtained in *E. haemastoma*, indicating the absence of lignin. The size of the epidermal cells is variable in one and the same seed. At the edges and ribs and around the hilum the height of these epidermal cells can be many times the breadth (macrosclereids). On the flat side of the seed they are more or less isodiametrical or even frequently elongated parallel to the surface of the seed. They are relatively small in *E. stellulata* (Pl. i, fig. 4) and *E. salicifolia*, and by comparison very large in *E. planchoniana*, *E. laevopinea* (Pl. i, fig. 1) and others. The outer cuticle is missing, or at the most just discernible as fragmentary remnants as in *E. oreades*, *E. kybeanensis*, *E. stellulata* and *E. piperita*. The inner epidermis is made up of small, thin-walled tabular cells. The number of layers of parenchymatous cells between the two epidermal layers changes not only with the species but also with the position on the seed at which the examination is made, that is to say, at ribs and corners, and particularly along the raphe there is always a larger number than on the flat side of the seed. There are a few layers only in *E. sparsifolia*, *E. salicifolia* and *E. oreades* (two to three), and they are numerous in *E. planchoniana* (six to eight).

Monoclinic crystals of primary calcium oxalate monohydrate (identical with the mineral whewellite) occur dispersed in the cells of the outer integument (excluding the outer epidermis). They are most plentiful in the raphe parenchyma just over the hilum, where they are arranged in vertical files accompanying the vascular bundle (Text-fig. 4). They are often also more numerous in the ribs. There is special significance in their localization in the inner epidermis.⁴ In some species (*E. piperita*, *E. pilularis*, *E. planchoniana*) they are missing or extremely rare on the raphe-free sides. In *E. stellulata* they occur only in the lower part of the seed. In *E. salicifolia* their occurrence is patchy. They are small and scarce in *E. oreades*, but scattered over the whole raphe-free sides. They are abundant in *E. macrorrhyncha* (Text-fig. 5) and *E. haemastoma* (Text-fig. 1). In *E. sparsifolia* nearly every epidermal cell accommodates a large, well-developed crystal. In *E. muelleriana* many of the crystal-bearing cells have irregular, uneven wall thickenings which partly surround the crystals or enclose them completely (Text-fig. 2). Furthermore, some of these crystals in the hilar region have an additional cellulose membrane as an envelope (Text-fig. 3).⁵

This discontinuous presence or complete absence of calcium oxalate crystals in generally unmodified cells of the inner epidermis of the outer integument is likely to have taxonomic significance. As will be indicated later, this epidermis in other sections of the genus is formed of cells strongly thickened at the base, each carrying one or more crystals. In the Angiosperms the occurrence of this "crystal epithelium" is widely distributed, especially in the more primitive families, and is generally considered in the phylogenetic sense as a primitive character. Within the Renantherae the inner epidermis has, to a greater or lesser extent, lost its character as a "crystal epithelium".

³ Strictly, one ought to speak of the derivative of the outer integument, but to simplify discussion we shall refer to the two parts forming the seed coat as inner and outer integument.

⁴ Isolation of the inner epidermis exposing larger areas is necessary to obtain correct information about their distribution. It is also important to compare raphe-free sides above the hilar region because crystals are always present in the vicinity of the bundle, hilum and micropyle.

⁵ Blakely's Series Pseudo-Stringybarks comprises three species: *E. pilularis*, which we found crystal-free, *E. muelleriana*, where about half of all epidermal cells are crystal-bearing, and *E. wardii*. Of the last-named we had only a few seed fragments from the holotype for examination, revealing about the same frequency of crystals in somewhat irregularly thickened cells as in *E. muelleriana*, though in a lesser degree. The hilar region could not be investigated.

As a whole the outer integument represents the "pigment layer", and the ripe seed displays, due to this, a brown to black colouring. There are dark-coloured compounds, giving tannin reaction,⁶ impregnating the walls and filling as amorphous deposits the lumina of the epidermal cells, the parenchyma and the chalaza cork tissue (Text-fig. 18). They are probably closely related to the phlobaphenes (anhydrides of tannin) of the bark. In the White Mahoganies (*E. triantha* and *E. carnea*) impregnation of the walls of the outer epidermis is weak or even absent. On thin sections they appear ivory white and give a strong phloroglucin reaction which is partly masked in other species, due to the brown colouring by tannin impregnation.

As the result of this study of the structure of *Eucalyptus* seed we consider the raphe as part of the outer integument and not—as it still is described in some recent literature—as the fusion of the funicle with the testa. The parenchyma and the epidermis of the raphe have the same structure and contents as the rest of the outer integument. Thus in the Renantherous seed coat the same sclereid epidermis covers the raphe as well as the remainder of the seed. Where the inner wall of the outer epidermis cells is mucilaginous, as in *E. marginata*, or where palisade-like thin-walled epidermal cells filled with solid dark-coloured material occur in other systematic groups (*E. maculata*, *E. citriodora*) we find them also unchanged over the raphe in wall structure and contents.

The hilum is a clearly seen scar surrounded by a raised rim of sclereids. There is no protective layer covering the exposed surface, which is neither cutinized nor suberized, nor covered by a cuticle. Thus the exposed cells wear away, the surface breaks up and in the course of time the hilum becomes more or less hollowed and air-filled. This may rather retard than facilitate the intake of water for germination.

From the hilum an amphicribal vascular system with helically thickened tracheids extends in the expanded raphe parenchyma right up to the chalaza (which is not externally visible) where it finally spreads out (Pl. i, figs. 3, 4, and Text-fig. 19, 1). Integumentary bundles, i.e. a system of strands branching off the raphe bundle and traversing the outer integument, do not exist.

The Inner Integument.—This occurs immediately below the outer integument. In ripe seeds the median cuticle delimiting the two integuments is resorbed. In the genuine Renantherae the inner integument is two-layered, being formed of both epidermal layers alone. The cells are tabular and without intercellular spaces. In contrast with species in Blakely's two series, Steatoxylon and Myrtiformes, in all remaining series of the Renantherae-Normales this integument has not been resorbed in the course of ripening. It is suberized⁷ throughout (Pl. i, figs. 1, 3, 4), the walls being not merely impregnated with fatty substances. If after previous treatment with Eau de Javelle, tests are made with zinc chloroiodide, brown-coloured delicate suberin lamellae separate themselves from the primary cellulose walls which appear blue-coloured. The walls are in addition more or less impregnated with tannin-like material.

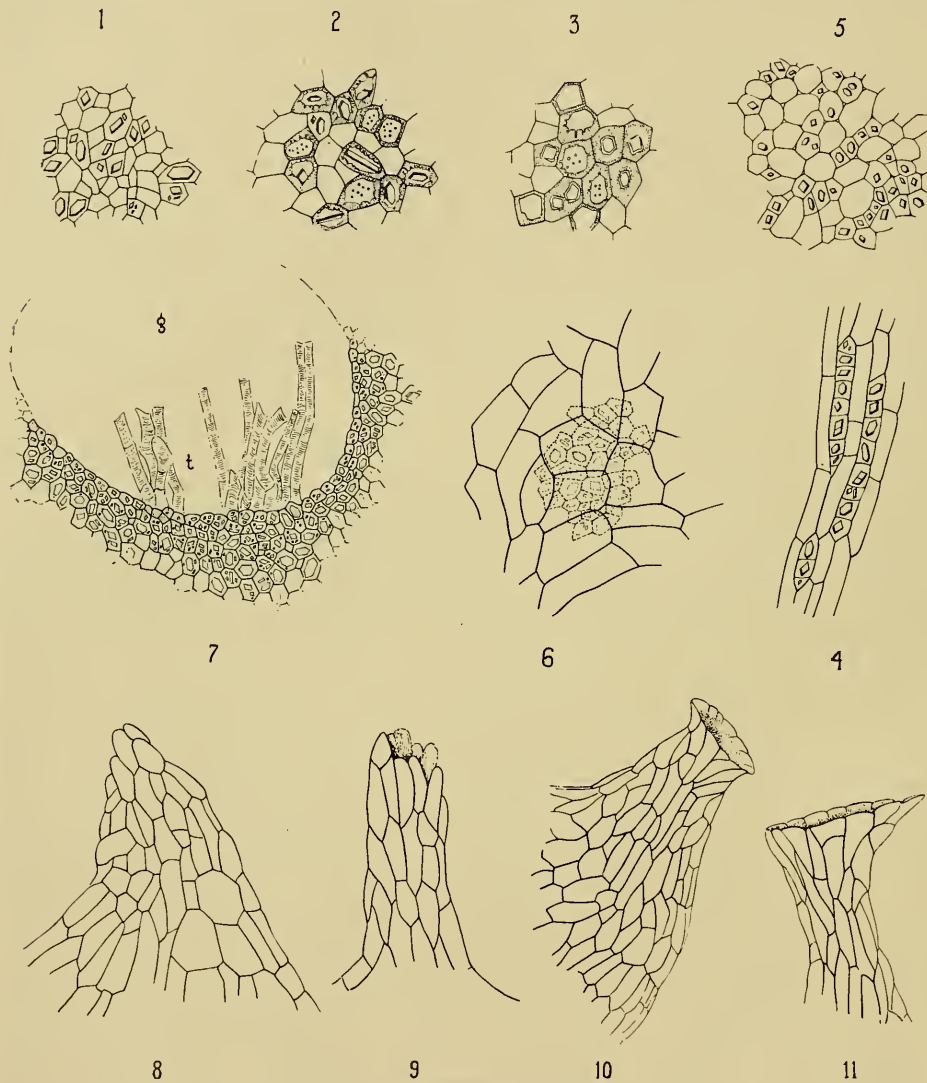
The shape and size of the micropylar end of the inner integument vary considerably within the same species. There is either a blunt or pointed tip without any sign of an aperture, or the apex is lengthened to form a cylindrical or conical tube, the endostome, with a straight or somewhat funnel-like dilated rim. The capillary canal is blocked by reddish-brown material which also fills the surrounding cells (Text-figs. 8, 9, 10 and 11). Thus there is little chance of an appreciable intake of water or nutrients through the endostome to the embryo once the suberization is completed.⁸

⁶The microchemical test for tannins in the wide sense of the term (which comprises very different substances) is generally limited to ferric salts staining green or blue. This of course does not reveal much about the precise nature of these compounds.

⁷Besides the fat-staining compounds which can give ambiguous results, Hoehnel's cerin acid reaction was used to test the suberin lamellae.

⁸The exostome is occluded, most of the species showing—*sub lente*—a faint short fissure or ridge between hilum and the micropylar region which could be considered perhaps as the exostome suture. However, this needs further investigation. But in view of the fact that moisture is readily absorbed by the whole cuticle-free epidermis it is of secondary importance for germination as to whether or not the intake of water is effective through hilum and micropyle.

Netolitzky's opinion is that the main role of cork tissues and cuticles in Angiosperm seed coats lies in the prevention of emigration of crystalloids (formed by mobilization of the colloids during germination) from the embryo.



Text-figs. 1-11.

1-7: Monoclinic crystals of calcium oxalate monohydrate, ca. 180 \times .

Fig. 1, 2, 5, 6, 7: crystal-bearing inner epidermis of the outer integument in surface view. 1: *Eucalyptus haemastoma*; 2: *E. muelleriana*; 5: *E. macrorrhyncha*; 6: *E. brachyandra*, the crystal epithelium is seen lying beneath the outer epidermis; 7: *E. guilfoylei*, the crystal epithelium is viewed from the inside of the seed. Through the gap (g) in the crystal layer (in the chalaza region) the tracheary endings (t) of the raphe bundle are seen.

Fig. 3: *E. muelleriana*, crystals with cellulose envelopes in thick-walled cells.

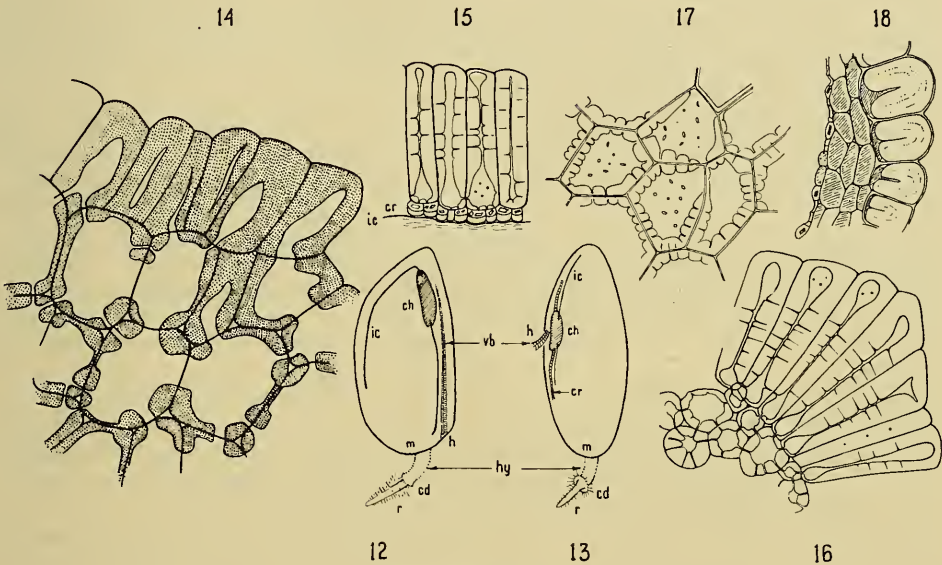
Fig. 4: *E. muelleriana*, crystals in short files accompanying the raphe bundle.

8-11: Some endostome forms, ca. 180 \times . 8: *Eucalyptus muelleriana*; 9: *E. oreades*; 10: *E. salicifolia*; 11: *E. gigantea*. The forms are variable within the same species.

At the inner limit of the inner integument (and therefore of the testa in general) an inner cuticle is encountered which is rather conspicuously developed and forms rib-like projections between the nucellus epidermal cells (Pl. i, fig. 1), so that in

surface view this cuticle simulates cellular structure. It is the product both of the inner integument and nucellus where their surfaces are in contact, and therefore of double origin. However, the formation of this cuticle is suppressed in the chalaza region.

The chalaza is reckoned also as part of the testa. It is the parenchymatous tissue where nucellus and integuments merge. It is the pathway for nutrients to the integuments and through the gap in the inner cuticle to the embryo and its associated tissue. Once the supply to the embryo is completed this gap is closed by suberization of neighbouring chalaza tissue and by filling the cork cells with dark-coloured solid material (Pl. i, figs. 3 and 4). The suberization is of the type of the inner integument, therefore there is an inner suberin lamella alongside the cellulose wall. It must be noted, however, that this "chalaza cork" is not of uniform origin, because not only do the cells of the chalaza *sensu stricto* undergo suberization, but partly the neigh-



Text-figs. 12-18.

12-13: Diagrams of germinating seed. 12: *Eucalyptus dives*; 13: *E. microcorys*—*cd*, clinging disc; *ch*, chalaza cork; *cr*, crystal layer; *h*, hilum; *hy*, hypocotyl; *ic*, inner cuticle; *m*, micropylar region; *r*, radicle; *vb*, vascular (raphe) bundle.

14-17: Chaff structure. 14: *E. laevopinea*, transv. section; 15: *E. microcorys*, transv. section of chaff with ventral hilum; 16: *E. microcorys*, transv. sec. of chaff with basal hilum; 17: *E. guilfoylei*, tang. sect. of outer epidermis of chaff with ventral hilum showing the blind pits in the outer wall.

18: *E. fraxinoides*, transv. sect. of the outer integument, the phlobaphene-bearing cells (pigment layer) shaded. All sections *ca.* 180 \times .

bouring tissue of the nucellus is also involved—often, indeed, the latter forms the major portion of the whole "chalaza cork". Furthermore, the suberization extends also into the raphe parenchyma and adjoins the suberized inner integument (Pl. i, fig. 4), forming finally (in surface view) a large circular, oval or elliptical, dark-coloured disc above the smaller cuticular gap. With some care it is possible to extract the inner integument with the adherent chalaza cork revealing its size and shape.

It is beyond the limits of the present study to make an extended histochemical and structural investigation of the embryo and its supporting tissues and the alterations they undergo during the maturation of the seed. It seems, however, that nucellus and endosperm also in some circumstances can throw light on taxonomic relationships.

Therefore a few remarks about the final stage of these parts as seen in the mature seed are justified.

In the resting seed there is a more or less broad remnant of the nucellus tissue, especially in the chalaza region, the cells of which are empty and largely obliterated so that without application of swelling media the cellular structure in general cannot be distinguished (Pl. i, figs. 1, 3, 4). The endosperm is resorbed, except in the micropylar region, where an intact layer one cell thick and rich in proteins is retained (*E. muelleriana*, *E. alpina*, *E. sparsifolia*, *E. planchoniana*, *E. oreades* and others). Testing the germination residue of *E. planchoniana* with Millon's or Raspail's reagent reveals that these proteins have not been used during the germination. They represent a reserve surplus.

Finally, the embryo is itself covered by a cuticle which, however, does not at this stage form a tough skin but rather a semisolid envelope on which oil drops from the embryo dislocated by sectioning remain attached. In the resting seed, therefore, it has not reached its final consistency and it separates easily from the embryo epidermis. The seed being exalbuminous (without endosperm) the food reserves for germination are stored in the large embryo (cotyledons and hypocotyl) which fills completely the cavity enclosed by the seed coat. Storage substances are fats (in the form of oil droplets) and proteins (aleurion grains).⁹

With the exception of the two series Steatoxylon and Myrtiformes the testa structure described above is uniform in principle in all the series and subseries of the Renantherae-Normales, almost all of which have been investigated.¹⁰ In these groups the following species were examined: *E. pilularis*, *E. muelleriana*, *E. umbra*, *E. triantha*, *E. laeopinea*, *E. macrorrhyncha*, *E. alpina*, *E. sparsifolia*, *E. obliqua*, *E. gigantea*, *E. planchoniana*, *E. oreades*, *E. fraxinoides*, *E. kybeanensis*, *E. pauciflora*, *E. stellulata*, *E. salicifolia*, *E. dives*, *E. tasmanica*, *E. piperita* and *E. haemastoma*. There is little doubt in assuming that Blakely's categories, series and subseries, are essentially homogeneous. On this basis, therefore, the material examined satisfactorily represents 94 species.

In summary, the following points are distinct and characterize the testa of the Renantherae-Normales:

1. The outer integument consists of several layers, its outer epidermis is formed of sclereids, its inner epidermis does not form a specific crystal layer.
2. The inner integument persists, is two-layered and suberized.

Whether this structure is exclusively confined to this group of *Eucalyptus* and may be used as an index of relationship or whether it appears also in other systematic groups as the result of convergent development will not be fully apparent until the investigation of all the remaining sections in the genus is complete. For the moment we are able to point out that, for example, the testa of *E. patens* and *E. diversifolia* (Sect. Renantheroidae) and that of *E. jacksoni* (Sect. Macrantherae) are in all histological and morphological details precisely that of the pattern in the Renantherae-Normales.¹¹

Relating structure with function of the seed coat, it is obvious that the closely-packed epidermal sclereids confer high mechanical protection, while the biological function of the tannin deposits in the outer integument no doubt lies first of all in the chemical protection against decay. The strongly reduced permeability resulting from the suberized tissues (inner integument, chalaza) and the inner cuticle, all enclosing the embryo, indicates that in the mature seed the embryo has attained a far-reaching physiological independence from its coat.

⁹ We have examined the aleurion grains in the embryo of *E. marginata*. They include numerous globoids and one druse (cluster crystal) of calcium oxalate each. Treatment with picric acid and eosin also reveals a protein crystalloid (stained bright yellow) in the amorphous protein substrate (stained red). Thus they represent the most highly differentiated form of aleurion grains.

¹⁰ Only the Subseries Seminudae has not been examined. The four species belonging to this group are considered to be hybrids and authentic seed could not be obtained.

¹¹ It seems unlikely because of basic differences in testa structure that *E. jacksoni* and *E. diversicolor* are closely related. This is contrary to Blakely's view since he places them in the same Subseries, Inclusae.

In some respects the testa as a whole may be compared with the (outer) bark. The suberized inner integument is a dead tissue and excretion of oxalate crystals and phlobaphenes into the outer integument excludes its cells from metabolic activities.

The series *Steatoxylon* and *Myrtiformes*, both of which Blakely places in his *Renantherae-Normales*, present a quite different type of testa.

E. microcorys, of the monotypic series *Steatoxylon*, has flat, bifacial somewhat elliptical seeds. The coat is much reduced in thickness. The inner integument is in the course of the ripening process resorbed and is missing altogether in the mature seed (Pl. i, fig. 6). Only in the chalazal region, in the corner between the crystal layer and the inner cuticle, do a few non-suberized cells still appear. The outer epidermis is formed of thin-walled cells whose outer wall is often sunken (concave) or even torn and whose anticlinal somewhat wavy walls are partly separated from each other so that the seed surface appears somewhat shaggy. The inner epidermis forms a typical crystal layer in which each cell has a thickened basal wall and contains one large crystal enveloped in a cellulose membrane and some smaller ones. All cell walls of the integument give the tannin reaction with ferric salts. The major portion of the nucellus remnant is obliterated. Traces of endosperm are still seen, especially in the chalaza region, where, as with the nucellus remnant which also occurs there, cellular structure can be perceived. Here one can also see that suberized cells of the nucellus remnants contribute in a very high degree to the formation of the "chalaza cork" (Pl. i, fig. 5).

E. microcorys is distinguished therefore from the normal *Renantherous* type described above in the following ways:

1. The outer integument, except the raphe side, is only two-layered. The outer epidermis is composed of thin-walled cells which are often torn and separated from each other. The inner epidermis forms a typical crystal layer.
2. The inner integument is resorbed.

The other exception appears in the series *Myrtiformes*, of which *E. deglupta*, *E. brachyandra* (Text-fig. 6 and Pl. i, fig. 9) and *E. raveretiana* were examined. The structure of the testa is in principle like that of *E. microcorys* except that the epidermal cells are of lesser depth, more elongated, with straight anticlinal walls, remaining attached to one another, and the outer wall is not torn. The inner periclinal walls of the crystal cells are only moderately thicker than the outer walls. The crystals are not enveloped in a cellulose membrane.

SECTION RENANTHERAE.

Blakely has erected a section *Renantherae* containing two series which are not in the section *Renantherae-Normales*, i.e. the *Occidentales* and the *Ochroxylon*. Of the five species of the *Occidentales* we were able to examine *E. marginata*, *E. staerii* and *E. sepulcralis*, which agree with one another well and are characterized by the following features:

The outer epidermis has a cuticle-free, thick, strongly lignified outer wall which on the inside has a thick mucilaginous lamella extending along the side walls almost to the thin base wall of the cells (Pl. i, fig. 2). This mucilaginous layer is strongly impregnated with tannin material, swells in water displaying lamellations, and contracts in alcohol.¹²

The parenchyma of the outer integument is strongly developed and filled with tannin deposits. In *E. staerii* crystals are scattered over the whole inner epidermis, while in *E. marginata* and *E. sepulcralis* they are confined to the hilar region.

The inner integument is suberized and in general two-layered, in *E. staerii* in some places three- or four-layered. The suberin lamellae display a fine granulation which

¹² Staining to test the nature of this mucilage is masked by tannin impregnation. This can be removed by Eau de Javelle, whereupon chloriodide of zinc gives a dark blue colour and cuprammonia a light blue. This points to cellulose mucilage. On the other hand, ammoniacal ruthenium sesquichloride stains deep red, indicating the presence of pectin mucilage. It seems, therefore, that both kinds are present, if staining alone is a sufficient proof. There is no specific microchemical reagent for pectin.

was also observed in some of the Renantherae-Normales species, though to a lesser degree.

The nucellus remnant for the most part has lost cellular structure, but here and there it can be distinguished (Pl. i, fig. 2).

This type of testa appears as a variant of the type of the Renantherae-Normales. The mucilaginous wall component of the epidermal cells does not represent a significant structural variation, since in an alcohol preparation the outline of the total thickening (cellulose plus mucilage layer) is exactly the same as that of the epidermal sclereids of the Renantherae-Normales. It is irrelevant to this study—and therefore has not been investigated—as to whether this mucilage as such has been deposited upon the cellulose walls or whether it is due to subsequent partial conversion of the walls into mucilage.

The structure of *E. guilfoylei*, of the monotypic series Ochroxylon, is quite different (Pl. i, fig. 10). The outer integument of the flat seed is, viewed from the raphe-free side, formed from the two epidermal layers only. The cuticle-free outer epidermis is made up of thin-walled cells with walls impregnated with brown material which often adheres also to the wall in the form of irregular lumps which do not give any tannin reaction with FeCl_3 . The inner epidermis is a typical crystal epithelium. The inner integument is resorbed. The structure has therefore nothing in common with the large bulk of the Renantherae and resembles much more in general *E. microcorys*, with which it also has in common the cellulose envelopes of the crystals.

To sum up, we can therefore say that on the basis of testa structure the three series, Ochroxylon (*E. guilfoylei*), Steatoxylon (*E. microcorys*) and Myrtiformes (*E. deglupta*, *E. brachyandra* and *E. raveretiana*), do not conform to the combined group Renantherae and Renantherae-Normales. This evidence conforms with Maiden's view of the relationship of *E. microcorys*. He says (I, 262): "This species appears to stand by itself amongst the Renantherae to a greater extent than any other members of that group." To place correctly *E. microcorys* and the other species mentioned many more factors must be considered. This will be done at the conclusion of the anatomical study of the seed of the remainder of the genus.

There is also still another difference which separates these three series from the remainder of the Renantherae. This is the close placing of hilum and chalaza and consequently a different pattern of attachment of the seed and its vascularization.

The seed of the Renantherae is of somewhat irregular form, but it always displays about four or five raised ribs which spread out from a clearly marked basal hilum, that is at or near the base of the seed. As this type of seed originates from an anatropous ovule, the hilum lies in the vicinity of the micropyle through which the hypocotyl emerges. Therefore the germinating seed shows the hypocotyl close to the hilum at the base of the seed which is more or less perpendicularly attached to the placenta, while the chalaza occupies the other end of the seed (Text-fig. 12). The vascular bundle diverging from the placenta as a single collateral strand into the outer integument ramifies sooner or later in numerous more or less arcuate ascending, not anastomosing, amphicribal branches which fan out over the chalaza, where they terminate (Text-fig. 19, 1). In transverse section they are seen arranged in a flat arc facing the chalaza (Pl. i, figs. 3, 4).

In the three series which do not conform to the general Renantherous pattern the seeds are bifacial with a ventral hilum, that is in the middle or perhaps a little towards the upper end of the one side, and close to the chalazal region, or even within it (as in *E. microcorys*).¹³ Thus the germinating seed shows the hypocotyl at the lower (micropylar) end and the hilum quite distant from it near the middle of the seed which is attached like a snield to the placenta (Text-fig. 13). This leads to

¹³ Discussing the ventral hilum of the Corymbosae, Maiden (VII, 95) mentions that after removal of the testa the scar beneath (this means probably on the inside) "is often larger and more distinct and definite in shape to that observed on the testa". This "scar beneath" is of course the chalaza cork and has nothing to do with the scar of the hilum. The occurrence of hilum and chalaza face to face, or nearly so, is characteristic for seed with a ventral hilum, as in *E. microcorys*.

the conclusion that such a seed originates from another type of ovule, the hemitropous. But further investigation must be undertaken when suitable material for ontogenetic study is available to see whether this structure is not perhaps the result of change by growth of an ovule also originally anatropous. Goebel (1933) has illustrated by different examples a change by growth before or after fertilization resulting in an alteration of ovule shape, for example one originally anatropous becomes campylotropous (Geraniaceae) or an anatropous ovule changes to hemitropous (*Torenia asiatica*).

Through whatever phases these ovules may pass during their development the investigation of flower buds of *E. microcorys* just before anthesis shows that they are already of hemitropous shape (Pl. i, fig. 7). The simultaneous occurrence of anatropous ovules (Pl. i, fig. 8) will be discussed in connection with the chaff structure.

The phylogenetic and taxonomic importance of the ovule shape and structure has been emphasized often by many authors (see Warming, 1913), though in higher groups (families, etc.) different types may occur. In the Myrtaceae, for instance, the bitegmic anatropous ovule is dominant, but campylotropous ones occur too, and *Jambosa caryophyllus* is quoted as having only one integument. Thus it is not surprising that a genus as large as *Eucalyptus* has more than one ovule type.

Due to the ventral position of the hilum and in contrast with the elaborately ramified raphe bundle of the Renantherae, the conducting tissue of these three series is greatly reduced in extent and ramification, and its xylem built up by short, wide, irregularly shaped and sometimes branched tracheids with truncate ends, while in the Renantherae the tracheids are slender and long (Text-fig. 19, 1-6).

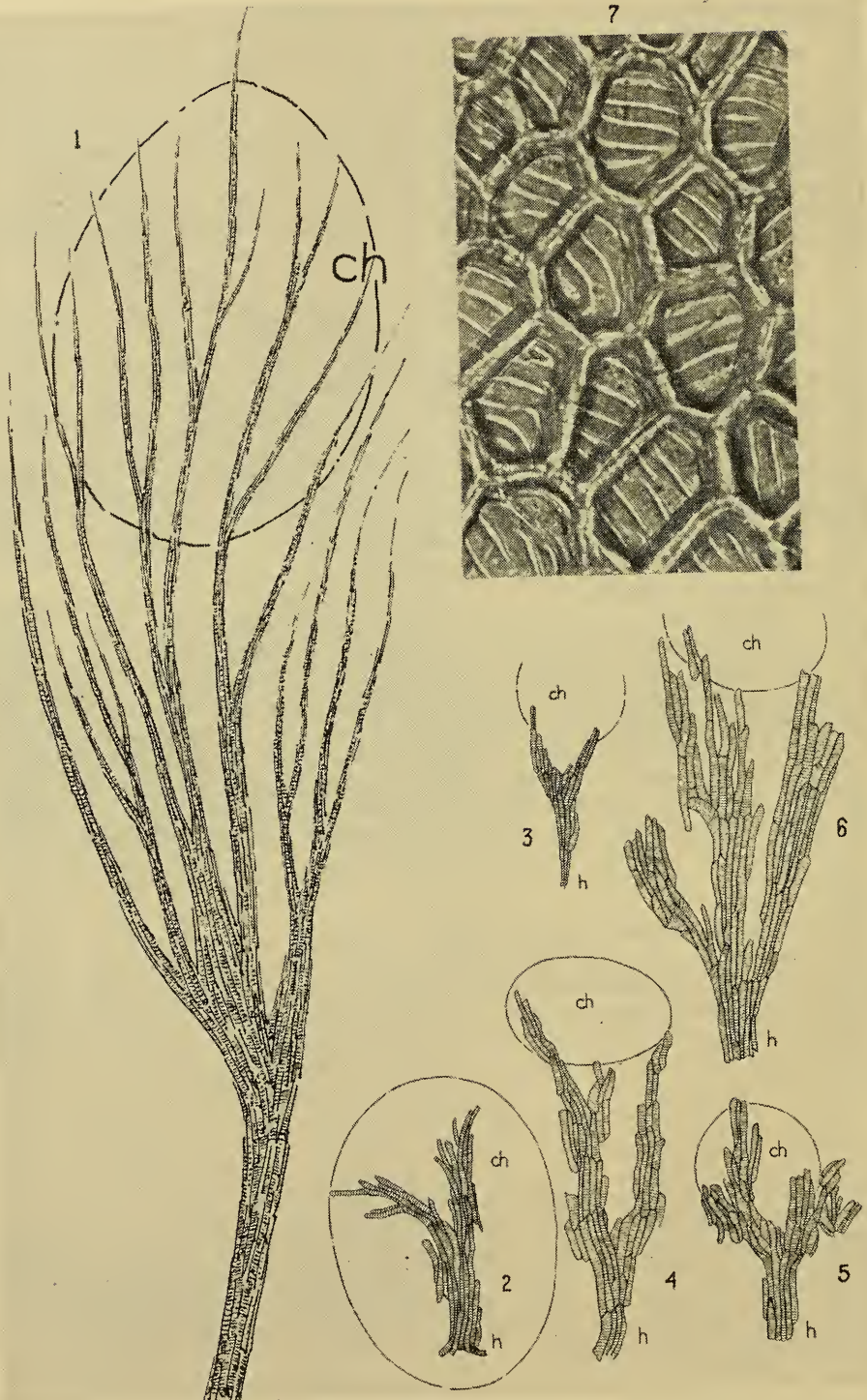
It is a well established fact that vessels in vegetative parts have undergone phylogenetic modifications culminating in the formation of short, wide vessel members with truncate ends (Bailey, 1944). Tracheids likewise have become shorter, though in a lesser degree. But too little is known about their structure and evolutionary trends in raphe bundles. Kuehn (1928) has investigated the course of intraseminal (= integumentary) bundles of the Angiosperms. Though this vascularization type does not occur in our species—there are only raphe bundles—her results are of great interest: the intraseminal vascular system has arisen independently in unrelated families, primitive and high ranking, and some families (Oleaceae, Leguminosae, Compositae, etc.) comprise genera with and without such bundles. This leads to the conclusion that they are of little phylogenetic significance. However, this does not mean that the vascularization pattern is of no taxonomic value for generic or infrageneric grouping.

Kuehn has not examined the Myrtaceae. Concerning the structure of intraseminal bundles she remarks only that they are built up by helically or annularly thickened tracheids. To our knowledge no other evidence in connection with taxonomic evaluation of seed vascularization has been published and its significance in the genus *Eucalyptus* will show up when all sections have been investigated. It already seems likely, however, that the two vascularization patterns are closely connected with the ovule type.

Finally, a few remarks on the nature and structure of the chaff seem appropriate.

We have examined these sterile bodies in *E. muelleriana*, *E. laevopinea*, *E. alpina*, *E. obliqua*, *E. planchoniana*, *E. oreades* and *E. fraxinoides*. They are in principle in perfect conformity in all respects. The outer epidermis is similar to that of seed but encloses a tissue which may be best termed sclerotic parenchyma. On thin sections it has some resemblance to angular collenchyma in so far as the wall thickenings are apparently only in the cell corners while the side walls appear thin due to the very wide pits (Text-fig. 14). All cells are strongly lignified and filled with phlobaphenes staining inky black with ferric chloride. A disorganized vascular bundle can be located embedded in this tissue.

The chaff of *E. microcorys* is of a strikingly different structure. While the seed epidermis is formed by thin-walled cells (Pl. i, fig. 6), the chaff epidermis is exactly of the Leguminosae type, that is to say, built up by palisade-like sclereids many times deeper than wide (Text-figs. 15, 16). The bodies forming this chaff are of very



Text-fig. 19.

1-6: raphe bundles, ca. 130 \times . 1: *Eucalyptus dives*; 2: *E. microcorys*; 3: *E. brachyandra*; 4: *E. raveretiana*; 5: *E. deglupta*; 6: *E. guilfoylei*. (ch, chalaza region; h, hilum.)
 7: *E. guilfoylei*, tang. sect. of the outer epidermis of chaff with basal hilum showing scalariform thickenings on the outer wall (photomicrograph, ca. 400 \times).

different size and shape, but they can be placed in two groups: small, narrow ones with a basal hilum, and larger, flat ones, often glossy, with a ventral hilum.

To trace their origin an ontogenetical investigation is needed. We could examine only flower buds just before anthesis and this revealed the presence of two kinds of ovules in the ovary. Firstly, the fertile, at this stage already hemitropous ovules which have two distinct two-layered integuments with three cuticulae: an outer covering the surface, a median between the two integuments and an inner one delimiting the inner integument from the nucellus (Pl. i, fig. 7).¹⁴ These ovules develop after fertilization into seeds losing during maturation the outer and median cuticle and the inner integument.

Besides these fertile ovules there are many sterile, atropous ovules of prismatic shape, with basal hilum and distal micropyle. They are built up by a parenchymatous tissue with a procambial strand in the axis. At its apical end this parenchyma encloses a cavity leading into the micropyle and lined with a cuticle. The outline of this cavity varies with the section and is seen as either empty or containing a small, more or less isolated group of parenchyma cells (Pl. i, fig. 8). If we interpret this cuticle as the inner one, then the outer parenchyma represents a modified integumentary tissue and that within the cavity a modified nucellar tissue.

It is probably correct to assume that these sterile ovules mature finally into chaff with a basal hilum. Thus this chaff is already predetermined before anthesis. In conformity with the ovules from which it originates it shows a poor histological differentiation, the tissue consisting mainly of the epidermis and the parenchyma, both strongly sclerified and lignified (Text-fig. 16).

The chaff with a ventral hilum develops probably from fertile but not fertilized ovules. The hemitropous shape not only conforms with this suggestion, but also the richer tissue differentiation does too: outer epidermis, crystal layer, inner cuticle and an obliterated nucellus remnant, but of course no embryo (Text-fig. 15).

E. guilfoylei displays two similar kinds of chaff, of which we mention only the different architecture of their epidermal cells. All walls, except the inner periclinal ones, have conspicuous thickenings which are pitted even on the outer walls (blind pits) in chaff with a ventral hilum (Text-fig. 17), while in chaff with a basal hilum they are scalariform or scalariform-reticulate (Text-fig. 19, 7). As mentioned earlier, the seed epidermis of this species is thin walled (Text-fig. 10).

Thus, not only the seed coat but the chaff structure too deserves consideration and may contribute valuable information for taxonomic problems.

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¹⁴The outer cuticle is a single membrane, the two others are of double nature, as can be seen where the two integuments separate from each other (Pl. i, fig. 7) or the nucellus separates from the testa (Pl. i, fig. 8).

EXPLANATION OF PLATE I.

Cork tissue and cuticles brown.

1: *Eucalyptus laevopinea*, part of transv. sect. of seed. 2: *E. marginata*, part of transv. sect. of seed. The mucilage layers of the epidermis (*mu*) swollen by water absorption. Nucellus (*n*) partly crushed. 3: *E. tasmanica*, part of transv. sect. through the raphe-chalaza region at the level of the cuticula gap (the inner cuticle, *ic*, ending blind in the chalaza cork). 4: *E. stellulata*, part of transv. sect. through the raphe-chalaza region, somewhat below the cuticula gap (the inner cuticle, *ic*, is running through). The tracheids (*t*) of the amphicribal raphe bundle fan out over the suberized chalaza (*chc*). 5: *E. microcorys*, longit.-radial sect. through the raphe-chalaza region and the cuticula gap showing the course of the raphe bundle (*rb*) and the gap in the crystal layer (*cr*) and inner cuticle (*ic*). 6: *E. microcorys*, part of transv. sect. of seed. 7: *E. microcorys*, diagram and micropyle of the hemitropous ovule showing the three cuticulae (*oc*, *mc*, *ic*). 8: *E. microcorys*, long. sect. of a sterile atropous ovule with the single outer and the double inner cuticle. 9: *E. brachyandra*, part of transv. sect. of seed. 10: *E. guilfoylei*, part of transv. sect. of seed. All figures (except the diagram) ca. 180 \times . Figs. 7 and 8 microtome sections.

ch, chalaza; *chc*, chalaza cork; *cr*, crystal layer; *c*, embryo; *ec*, embryo cuticle; *en*, endosperm; *ic*, inner cuticle; *ie*, inner epidermis; *ii*, inner integument; *mc*, median cuticle; *mu*, mucilage; *n*, nucellus; *nr*, nucellus remnant; *oc*, outer cuticle; *oe*, outer epidermis; *oi*, outer integument; *pl*, placenta; *ps*, procambial strand; *rb*, raphe bundle; *t*, tracheids.